STATISTICAL ANALYSIS PLAN

Protocol PQ-421a-002 (HELIA)

AN OPEN-LABEL EXTENSION STUDY TO EVALUATE THE SAFETY AND TOLERABILITY OF QR-421A IN SUBJECTS WITH RETINITIS PIGMENTOSA (RP) DUE TO MUTATIONS IN EXON 13 OF THE USH2A GENE

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Name of Test Drug:	Ultevursen (QR-421a)
Phase:	2
Methodology:	Open-Label Extension
Sponsor:	ProQR Therapeutics Zernikedreef 9 2333 CK Leiden The Netherlands
Sponsor Representative:	
Document Date:	19-Oct-2022
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SIGNATURE PAGE

Protocol Title:	HELIA - An Open-Label Extension Study to Evaluate the Safety and Tolerability of QR-421a in Subjects with Retinitis Pigmentosa (RP) due to Mutations in Exon 13 of the USH2A Gene
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Version:	1.0 FINAL
Author:	
	Signature:_____
	Date:_____

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this prematurely terminated study.

I have discussed any questions I have regarding the contents of this document with the statistical author.

Sponsor Signatory:

Signature:_____

Date:_____

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MODIFICATION HISTORY

Current Version	Date	Amended by	Summary of Changes from Previous Version	Reason
1.0	19-Oct-2022	N/A	Original document	N/A

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1. ABBREVIATIONS

Abbreviation	Definition
AD	Analysis Description
AE	Adverse Events
AESI	Adverse Event of Special Interest
BCVA	Best Corrected Visual Acuity
CE	Contralateral Eye
CSR	Clinical Study Report
ETDRS	Early Treatment Diabetic Retinopathy Study
ICF	Informed Consent Form
ICH	International Council on Harmonization
LLVA	Low Luminance Visual Acuity
MedDRA	Medical Dictionary for Regulatory Activities
MH	Medical History
PT	Preferred Term
RP	Retinitis Pigmentosa
RTF	Rich Text Format
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD-OCT	Spectral Domain Optical Coherence Tomography
SOC	System Organ Class
TE	Treated Eye
TEAE	Treatment Emergent Adverse Event
TLF	Table Listing Figure format
WHO	World Health Organization

2. INTRODUCTION AND STUDY OBJECTIVES

2.1. Introduction

Study PQ-421a-002 (Helia) aimed to evaluate the safety, tolerability, and efficacy of ultevursen in subjects with Retinitis Pigmentosa (RP) due to mutations in exon 13 of the *USH2A* gene. This open-label, extension study allowed subjects who have participated in ultevursen clinical studies to continue to receive treatment or follow up, and to observe the long-term safety, tolerability, systemic exposure, and efficacy. This open-label extension study also allowed for treatment of the previously untreated contralateral eye (CE; if applicable).

2.2. Study Termination

In this context, the Analysis Description (AD) is abbreviated and describes the populations for analysis, data handling rules, statistical methods, and formats for data presentation that will be required for the close out of the study, after all subjects have completed the end of study visit and the database is locked.

The summary tabulations and listings that will be produced for the close out of the study, will provide the basis for the appropriate sections of the abbreviated clinical study report (CSR) or equivalent document.

3. STUDY DESIGN

3.1. Synopsis of Study Design

PQ-421a-002 is an open-label, extension study to evaluate the safety, tolerability, and efficacy of QR-421a in subjects with RP due to mutations in exon 13 of the *USH2A* gene. Subjects who participated in a preceding ultevursen clinical studies will be given the opportunity to participate in this extension study for continued dosing, provided the subject's benefit-risk assessment is positive, or for additional follow up.

Definition of the Treatment Eye and Contralateral Eye in Helia

Contralateral eye (CE)/Fellow eye is defined as follows: For subjects previously dosed (active or sham) in the preceding ultevursen study: the eye that was not selected as the treated eye (TE). For subjects that were not dosed at all: the eye with the worst BCVA at Helia Screening/Day1.

Treated eye (TE)/Study eye is defined as follows: For subjects previously dosed (active or sham) in the preceding ultevursen study: the eye that was selected as the TE. For subjects that were not dosed at all: the eye with the best BCVA at Helia Screening/Day1.

3.2. Analysis Groups by Eye

For analyses performed at the eye level, the TE and the CE will be grouped separately into the following analysis groups within each analysis set.

i. Ultevursen 180 µg/60 µg: a dosing regimen consisting of a 180 µg loading dose and a maintenance dose of 60 µg every 6 months during the HELIA study.

ii. Ultevursen 60 µg / 60 µg: a dosing regimen consisting of a maintenance dose of 60 µg every 6 months (without a 180 µg loading dose) during the HELIA study.
iii. All eyes: all TE or CE in the analysis set, depending on the eye for analysis

3.3. Analysis Groups by Subject

For analyses performed at the subject level, subjects will be grouped into the following analysis groups within each analysis set as follows. Subject-level analyses will include subjects who received the specified treatment in either eye:

- Ultevursen 180 ug/60 µg: receipt of a dosing regimen consisting of a 180 µg loading dose and a maintenance dose of 60 µg every 6 months in the HELIA study in the TE, CE or both.
- Ultevursen 60 ug/60 µg: receipt of a dosing regimen consisting of a 60 µg loading dose and a maintenance dose of 60 µg every 6 months in the HELIA study in the TE, CE or both. These subjects ***never*** received the ultevursen 180 ug/60 µg dosing regimen in any eye.
- All subjects: all subjects in the analysis set.

3.4. Study Procedures

Please refer to Section 8.0 of the study protocol for details regarding the study procedures.

3.5. Unmasking

This is an open-label study; blinding is not applicable.

4. POPULATIONS DEFINITIONS

The following analysis sets will be evaluated and used for presentation and analysis of the data.

Analysis sets defined by eye will be respect to each eye. For example, the Safety Analysis Set for the CE will be determined by measurements taken on the CE. The subject-level analysis sets consist of subjects who meet the criteria in at least one of the eye-level analysis sets.

Screening Analysis Set: Includes all subjects who signed the informed consent form.

Safety Analysis Set (SAF): Includes all subjects/eyes who received at least one dose of study drug in Helia in either eye. Subjects/eyes will be analyzed according to their actual treatment. The SAF will be defined at the subject level or the eye level, depending on whether the endpoint is to be analyzed at the subject level or the eye level.

The disposition data will be presented for all screened subjects. The SAF will be the primary set for the analysis of the safety parameters. The SAF will also be used for descriptive summaries of demographics, baseline characteristics, and ocular/non-ocular medical history.

5. STATISTICAL METHODS

5.1. Computing Environment

All information and analyses in the tables and listings provided will be performed using SAS statistical software Version 9.4 or later, unless otherwise noted. The Medical History (MH) and Adverse Events (AEs) will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 24.1. The Prior and Concomitant Medications will be coded using World Health Organization (WHO) Drug version Global B3 September 2021.

5.2. Definition of Baseline

Baseline is defined as the data most recently collected prior to the administration of study treatment.

5.3. Data Handling

5.3.1. General

Unless otherwise noted, the data reported in the listings will be as reported on the electronic case report form.

5.4. General Statistical Methods

All output will be incorporated into Rich Text Format (RTF) files and bookmarked PDFs, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate disposition and demographic characteristics.

For measurements of continuous variables, summary statistics of absolute values will be reported and will include n, mean, standard deviation, median, and minimum and maximum values. The number of missing values will be displayed in parenthesis next to ‘n’. Mean, standard deviation, median, will be presented with one more decimal place compared to the raw data, and minimum and maximum will be presented with the same number of decimal places as the raw data.

For categorical variables, summary tabulations of the number and percentage within each category of the parameter will be presented (as well as the number for missing data). Denominator for percentages is column N. Percentages will be rounded to one decimal place. Therefore, there may be cases where for instance the total of the percentages does not exactly equal 100%.

Where appropriate, data will be listed by eye (i.e., TE, CE).

In summary tables the study treatments will be presented in the following order: 'Ultevursen 180/60 µg', Ultevursen 60/60 µg', and 'All Subjects'.

5.5. Study Population

5.5.1. Subject Disposition

Subject disposition, including the number of screened subjects, the number of subjects failed screening, the number of randomized subjects, the number of subjects who received study treatment in the TE, the number of subjects who received study treatment in the CE, who completed the study and the number of subjects who discontinued from the study, along with the reasons will be summarized by treatment group.

A listing of subject disposition will be generated, including the reason for premature study or treatment discontinuation, if applicable.

A listing of screen failure data will also be provided.

5.5.2. Demographic and Baseline Characteristics

Demographic and baseline characteristics, including age as collected, gender, race, ethnicity, and baseline BCVA (ETDRS letter score for both TE and CE), will be summarized for the Safety Population by treatment group using descriptive statistics.

5.6. Safety – Adverse events

A treatment-emergent AE (TEAE) is defined as an event that was not present prior to administration of the dose of study drug (ultevursen or sham-procedure) and present after the dose or if it represents the exacerbation of an event that was present prior to the dose.

Adverse events will be summarized by treatment group separately as follows:

- For non-ocular AE:
    - by subject, therefore, in any tabulation, a subject contributes only once to the count for a given adverse event (System Organ Class [SOC] or Preferred Term [PT]); and
    - During the overall study period (i.e., up to the last visit for the last subject).
    - Time to onset will be calculated in hours, relative to the last dose received, irrespective of which eye received the last dose (i.e., TE or CE).
- For ocular AE:

- by eye frequencies, separately for the TE and the CE, therefore, in any tabulation, an eye contributes only once to the count for a given AE (by SOC or PT). AEs that are reported in both eyes (i.e., OU) will be reported individually by eye in the tabular summaries; and
- During the overall study period (i.e., up to the last visit for the last subject).
- Time to onset will be calculated in hours, relative to the last dose received in the eye experiencing the ocular AE.

Unless otherwise stated, any summary of any type of TEAEs which includes the SOC and PT of the events will order the SOC and the PTs within the SOC by descending incidence of PT.

The following summary tables will be produced separately according to the type of AEs (ocular/non-ocular where applicable) as defined above:

- Overview of Ocular Adverse Events for Treatment Eye during the overall study period (Safety Population)
- Overview of Ocular Adverse Events for Contralateral Eye during the overall study period (Safety Population)
- Overview of Non-Ocular Adverse Events during the overall study period (Safety Population)
- Summary of Ocular Treatment-Emergent Adverse Events for Treatment Eye during the overall study period by MedDRA System Organ Class and Preferred Term (Safety Population)
- Summary of Ocular Treatment-Emergent Adverse Events for Contralateral Eye during the overall study period by MedDRA System Organ Class and Preferred Term (Safety Population).
- Summary of Non-Ocular Treatment-Emergent Adverse Events during the overall study period by MedDRA System Organ Class and Preferred Term (Safety Population).
- SAEs
- AESIs

5.6.1. Partial and Missing Date/Time for Adverse Events

Imputation of missing/partial AE date/time will be done only to identify TEAEs.

AE onset dates

- Partially missing onset date/time will be imputed as follows:

- When only Day is missing:
  - If Month & Year of the onset date are the same as Month & Year of the first administration date, the imputed onset date will be imputed as the minimum of the first administration date/time and the AE resolution date (imputed if needed).
  - Otherwise, the missing day will be replaced by "1."
- When Day & Month are missing:
  - If Year of the onset date is the same as Year of the first administration date, the imputed onset date will be imputed as the minimum of the first administration date/time and the AE end date (imputed if needed).
  - Otherwise, the missing Day & Month will be replaced by "01 JAN."
- If a partial AE start date is consistent with the actual start date being on Day 1, and either the AE end date information is completely missing, or the complete or imputed AE end date/time information indicates that the AE did not end before Day 1, the imputed AE start date will be Day 1 in the following scenarios:
  - The AE start time is missing (the AE will be flagged as treatment-emergent following the first dose of study treatment)
  - The AE start time is after the date/time of first dosing of study treatment on Day 1 (the AE will be flagged as treatment-emergent following the first dose of study treatment)
  - The AE start time is before the time of first dosing of study treatment on Day 1, and imputing the AE start date as Day 2 instead of Day 1 would be inconsistent with the partially recorded AE start date (e.g., the calendar month for Day 2 is not the same as for Day 1), or with the complete or imputed AE end date/time (in this case, the AE will not be flagged as treatment-emergent following the first dose of study treatment).
- Missing start time will be imputed with the time of administration if the day of AE start date and administration are identical, or with 00:00 otherwise.

- Completely missing onset dates for AEs will be imputed by the first administration date and the AE will be considered as treatment-emergent unless the end date/time of the AE (imputed if needed) or the end year of the AE (if day and month are missing) is entered and is before the first administration date. If the end date/time is before the first administration date, the AE will not be considered as treatment-emergent.

AE end dates

- If Day only is missing, incomplete end dates will be replaced by the last day of the month if it is not resulting in a date later than the date of the subject's last visit. In the latter case, the date of the subject's last visit will be used to impute the incomplete end date.
- If Day & Month are missing, Day & Month will be replaced by 31DEC if it is not resulting in a date later than the date of the subject's last visit. In the latter case, the date of the subject's last visit will be used to impute the incomplete end date.
- If the AE end date is completely missing, the AE will be assumed to be ongoing on the date of the subject's last visit and the incomplete end date will not be imputed.

5.7. Other Data Listings to be Generated

The following by-subject data listings for the Safety Population will be provided:

- Subject Disposition
- Screen Failures (All Screened Subjects)
- Demographic and Baseline Characteristics (Safety Population)
- Non-Ocular Medical History (Safety Population)
- Ocular Medical History (Safety Population)
- Study Drug Administration of the TE (Safety Population)
- Study Drug Administration of the CE (Safety Population)
- BCVA Based on ETDRS (Safety Population)
- Low Luminance Visual Acuity (LLVA) (Safety Population)
- Spectral Domain Optical Coherence Tomography (SD-OCT) (Safety Population)
- Static Perimetry (Safety Population)
- Microperimetry (Safety Population)

- Ophthalmic exam (Safety Population)

- Non-Ocular Adverse Events (Safety Population)
- Ocular Adverse Events (Safety Population)
- Prior and/or Concomitant Medications (Safety Population)
- Laboratory results: Hematology, Coagulation, Chemistry and Urinalysis (Safety Population)

6. CHANGES TO PLANNED ANALYSES

As the study is prematurely stopped by Sponsor's decision, no formal statistical analysis will be produced. Only disposition, demographic and baseline characteristics, and overview of AEs will be summarized in tables. By-subjects data listings will be produced based on raw data.

7. REFERENCES

1. International Council on Harmonization, Statistical Principles for Clinical Trials (ICH E9)

8. APPENDICES

The shells of planned outputs are provided in a separate document (PQ-421a-002_List of Planned Outputs_V1.0 Final (2022-10-19)).